

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
	AD-A097056	
4. TITLE (and Subtitle)	5. TYPE OF REPORT & PERIOD COVERED	
Biological characteristics of some improved radioprotectors.	Scientific/Journal article	
	6. PERFORMING ORG. REPORT NUMBER	
7. AUTHOR(s)	8. CONTRACT OR GRANT NUMBER(s)	
DAVID E. DAVIDSON, JR. MARIE M. GRENAN and THOMAS R. SWEENEY		
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS	
Division of Experimental Therapeutics Walter Reed Army Institute of Research Washington, DC 20012	P612780.843.2400	
11. CONTROLLING OFFICE NAME AND ADDRESS	12. REPORT DATE	
US Army Medical Research & Development Command Fort Detrick, Frederick, MD 21701	10 1980	
	13. NUMBER OF PAGES	
	16	
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report)	
Walter Reed Army Institute of Research Washington, DC 20012	Unclassified	
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE	
16. DISTRIBUTION STATEMENT (of this Report)		
Approved for public release; distribution unlimited.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)		
Radioprotectors, Amino thiols, Sulfhydryl compounds, Phosphorothioates, Mice, Dogs, Rhesus monkeys, MEA, AET, WR 2721, WR 3689, WR 2529, WR 44923, WR 77913, WR 168643.		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number)		
<p>Analog of 2-mercaptoethylamine (MEA) and 3-mercaptpropylamine (MPA) have been synthesized which exhibit more potent radioprotective activity, improved tolerance and more prolonged protection in laboratory animals. The biological activity of 4 structurally diverse amino thiols and 1 non-nitrogen-containing thiol will be discussed; 1) S-2(3-methylaminopropylamino)ethyl phosphorothioic acid (WR 3689) which is better tolerated and has better oral protective</p>		

AD A 097056

DTIC FILE COPY

DD FORM 1 JAN 73 1473

EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

20. (Abstract) cont'd

activity in mice than WR 2721; 2) S-3(3-aminopropylamino)propyl phosphorothioic acid (WR 44923) which has more prolonged parenteral and oral activity than WR 2721; 3) Sodium hydrogen-S-(3-amino-2-hydroxypropyl) phosphorothioate (WR 77913) which is well tolerated and has provided exceptionally good protection to dogs; 4) 2-(2'-carbamidoethyl)amino ethanethiol (WR 2529) which has protected mice, monkeys and swine; 5) Sodium-4,4'-trithiobisbutane-sulfinate (WR 168643), an exceptionally well-tolerated non-nitrogen compound with good oral and parenteral protective activity in mice.

Each of these radioprotective compounds has been selected for further detailed preclinical investigation of safety and efficacy to determine whether it might be a candidate for use in protecting man against ionizing radiation. Their possible utility as an adjunct to clinical radiotherapy of tumors is also being considered.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Biological characteristics of some improved radioprotectors

David E. Davidson

Marie M. Grenan

Thomas R. Sweeney

Introduction

During the 14-year period between 1959 and 1973 the U.S. Army Medical Research and Development Command sponsored a coordinated Antiradiation Drug Development Program. The objective of this program was to develop a drug or combination of drugs which could be taken by military personnel or other populations to protect them from the effects of the ionizing radiations in a nuclear weapons attack.

During the program approximately 4400 compounds were chemically synthesized and tested in mice. The vast majority of these compounds were aminothiols. By 1973, Investigational New Drug Applications had been prepared on five compounds, and limited human tolerance studies were conducted on three of these.

When the Army program was started in 1959 the phenomenon of radiation protection by aminothiols and also by agents inducing hypoxia had been clearly demonstrated in a variety of *in vitro* and *in vivo* biological systems.^{2,8} Approximately 1500 compounds had been tested *in vivo*, and some 200 compounds had been reported as having activity.

The best of these were the aminothiols, and it was hypothesized that these protected by some mechanism other than by inducing hypoxia.¹ The ability of these thiols to scavenge radiation-induced free radicals had been described and this was proposed as a mechanism of action.¹ The binding of thiol protectors to sulfhydryl receptor sites had also been

described, and this was also proposed to be involved in the protective action.⁷

It had been determined that for optimal radioprotective activity among the aminothiol class of compounds, the essential structural features were a free sulfhydryl group or a potentially free sulfhydryl group separated by no more than three carbon atoms from a nitrogen functional group.⁶


In the chemical synthesis effort the program explored various sulfhydryl covering functions which were designed to be cleaved *in vivo* to release a free sulfhydryl group. The program examined well over 50 different sulfhydryl covering functions. Of these, the thiosulfate, phosphorothioate, disulfide, and thiazolidine covering functions produced the most interesting compounds (Table 1). The influence of various substituents on the nitrogen function were also studied. Both substituents on nitrogen and substituents on sulfur greatly modified the radioprotective activity and the pharmacology of the agents. The influence of substituents on the 2 or 3 carbon chain was also studied but these efforts were somewhat limited because relatively few main chain substitutions were possible which did not reduce or eliminate protective activity. There are, however, some exceptions to this, including one interesting compound which will be discussed below.

Radioprotective activity of MEA, AET and WR 2721

The best radioprotective compound from all standpoints which the Army program developed is the phosphorothioate designated WR 2721. This compound protected mice, dogs, and rhesus monkeys against x- or γ -irradiation, and protection

From the Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C.

TABLE 1

Analogues of Aminothiols	
	$R_1-NHCH_2CH_2S-R_2$
	$R_1-NHCH_2CH_2CH_2S-R_2$
Sulfur Covering Functions ($--R_2$)	
$-H$	Thiol
$-SO_3H$	Thiosulfate
$-PO_3H_2$	Phosphorothioate
$-SR$	Disulfide
	Thiazolidine

against neutron irradiation has been demonstrated in mice. Under ideal conditions in C57B1/6J mice, Yuhas demonstrated a dose reduction factor of 2.7 against 30-day mortality.¹² This is the highest DRF reliably reported for any single compound against the endpoint of lethality. Additionally, WR 2721 was far better tolerated in laboratory animals, providing an improved therapeutic index compared to earlier radioprotectors. The shortcoming of WR 2721 which limits its use for self-administration by military or other populations is its lack of adequate protective activity after oral administration.

Yuhas demonstrated that WR 2721 protects a variety of normal tissues, while certain solid animal tumors are not protected.^{13,14} This suggests possible

utility of radioprotectors as an adjunct to radiotherapy. For this application, lack of oral effectiveness would not appear to be a critical limitation and both preclinical and clinical trials are being conducted in several institutions.

In a series of meetings with representatives of the National Cancer Institute, five additional compounds have been selected which we believe show sufficient radioprotective activity in animals and which are sufficiently well tolerated in protective doses to be considered candidates for more detailed study in animal systems. Structural formulas for these compounds are presented in Table 2. Selected data will be presented in this report to highlight the radioprotective properties of each of these compounds which cause us to have a continued interest.

Some mouse testing data for MEA and for AET, two of the earlier radioprotectors, are presented in Tables 3 and 4. These compounds were tested in our laboratory under conditions comparable to those used to test the newer compounds which will be described below. In conducting these mouse tests, doses of radiation were given which were just sufficient to produce 100% mortality within 30 days in unprotected, vehicle control mice. For mice irradiated with a 250-kVp GE Maxitron x-ray unit, this dose was 800-850 rads midline tissue dose. For γ -radiation from ^{60}Co or ^{137}Cs small animal ir-

TABLE 2
Structural Formulas of Selected Radioprotective Compounds




Compound Number	Structural Formula and Chemical Name	Synthesis (Ref.)
WR 2721	$NH_2CH_2CH_2CH_2NHCH_2CH_2SPO_3H_2$ S-2-(3-Aminopropylamino)ethyl Phosphorothioic Acid	Piper et al. (9) Southern Res. Inst.
WR 2529	 3-(2-Mercaptoethylamino)Propionamide <i>p</i> -Toluenesulfonate	Carroll et al. (4) Research Triangle
WR 3689	$CH_3NHCH_2CH_2CH_2NHCH_2CH_2SPO_3H_2$ S-2-(3-Methylaminopropylamino)ethyl Phosphorothioic Acid	Piper et al. (10) Southern Res. Inst.
WR 44923	$NH_2CH_2CH_2CH_2NHCH_2CH_2CH_2SPO_3H_2$ S-3-(3-Aminopropylamino)propyl Phosphorothioic Acid	Piper et al. (9) Southern Res. Inst.
WR 77913	 Sodium Hydrogen-S-(3-Amino-2-hydroxypropyl)phosphorothioate	Piper et al. (9) Southern Res. Inst.
WR 168643	 Sodium-4,4'-Trithiobisbutanesulfonate	Srivastava (11) and L. Field Vanderbilt University

TABLE 3
Radioprotective Activity of MEA^a (HCl) in ICR Mice

Radiation Dose	Drug Dose (mg/kg)	Route	Time (minutes)	Survivors	Percent Survival	Toxic LD ₅₀ (mg/kg)
800 rads (x-ray)	150 ^b	I.P.	15	13/20	65 (3 tox)	200
	75			3/20	15	
	37.5			1/20	5	
	0			0/20	0	
	150 ^b	I.P.	60	3/20	15	200
	0			0/20	0	
	300 ^b	Oral	30	0/15	0	700
	150			0/15	0	
	0			0/10	0	

^a 2-Mercaptoethylamine hydrochloride.

^b pH adjusted to 7.1 in physiological saline.

radiators, the doses were 950–1000 rads. These doses were biologically equivalent, and results obtained using the various irradiators available at various times were comparable. The mice used were ICR/HA males or females from the Walter Reed colony, weighing 25–30 g at the time of irradiation. Radiation dose rates were in the range of 50–200 rads per minute depending upon the radiation source. Unprotected control mice were always irradiated simultaneously with each protected group of 30–40 mice, and controls and protected animals were jointly housed after irradiation in cage groups of five. Prior to each radiation experiment an acute toxicity study was performed to obtain an estimate of the toxic LD₅₀ and to characterize the dominant features of toxicity through

observation and gross pathology. The vehicles used were tailored to each compound based on considerations of solubility and stability. All drug doses reported in this manuscript are corrected for salt content and are expressed as mg of free base per kg of body weight.

In our laboratory, the acute toxic LD₅₀ for MEA was 200 mg/kg I.P. and 700 mg/kg orally. At doses of MEA in the maximum tolerated or minimum toxic range we were usually unable to protect 100% of mice against a lethal dose of radiation. In the example shown (Table 3), 65% survival was obtained with some toxicity. At half the maximum tolerated dose, little protection (15% survival) was observed, indicating a low therapeutic index. This is a feature of virtually all of the earlier radiopro-

TABLE 4
Radioprotective Activity of AET^a (Br · HBr) in ICR Mice

Radiation Dose	Drug Dose (mg/kg)	Route	Time (minutes)	Survivors	Percent Survival	Toxic LD ₅₀ (mg/kg)
800 rads (x-ray)	225 ^b	I.P.	15	15/20	75 (1 tox)	250
	112.5			12/20	60	
	56.2			6/20	30	
	0			0/20	0	
	225 ^b	I.P.	60	10/20	50 (3 tox)	250
	0			0/20	0	
	250 ^c	Oral	15	13/15	87	600
	250		30	15/25	60	
825 rads (x-ray)	250		60	1/10	10	
	250		90	1/10	10	
	0		—	0/20	0	

^a δ , β -aminoethylisothiourea bromide hydrobromide.

^b pH adjusted to 7.4 in phosphate buffer.

^c pH 4.5 in distilled water.

tectors, i.e., high survival was observed only at doses very close to the maximum tolerated dose (MTD). Another feature of MEA was its short duration of action. In the example shown, survival at the maximum tolerated dose was only 15% at 1 hour. After oral administration, little or no protection was evident in terms of survival.

AET was somewhat better tolerated than MEA and it was often possible to obtain 100% survival at a maximum tolerated dose. In the example presented in Table 4, there was 75% survival at a just-toxic dose. At half the maximum tolerated dose (112.5 mg/kg) survival was 60%, indicating a slightly improved therapeutic index. At one-fourth the MTD, survival was only 30%. When AET was given 60 minutes rather than 15 minutes before irradiation, there was still some protection as evidenced by 50% survival in mice given 225 mg/kg I.P. Orally, AET had good radioprotective activity in mice. At an oral dose of 250 mg/kg, protection

was optimal at 15 minutes (87% survival), but by 1 hour or longer, protection was minimal.

By comparison, WR 2721 (Table 5) provided more potent protection, more prolonged protection, and better oral activity in mice than either MEA or AET. It was also far better tolerated. The I.P. LD₅₀ was 950 mg/kg and the oral LD₅₀ was 1500 mg/kg.

At the maximum tolerated I.P. dose (600 mg/kg), 100% protection could be obtained quite regularly. In the example shown, 100% survival was also observed at one-half and one-fourth the maximum tolerated dose. At one-eighth the MTD there was still 80% survival, and below that dose, protection was minimal. This represents a marked improvement in therapeutic index. The duration of protection by WR 2721 after I.P. administration was also greatly extended. Protection was well developed by 30 minutes and appears to be optimal between 1 and 2 hours. Survival of over 50% of the

TABLE 5
Radioprotective Activity of WR 2721 in ICR Mice

Radiation Dose	Drug Dose (mg/kg)	Route	Time	Survivors	Percent Survival	Toxic LD ₅₀ (mg/kg)
825 rads (x-ray)	600 ^a	I.P.	15 minutes	15/15	100	950
				15/15	100	
				15/15	100	
				12/15	80	
				1/10	10	
				0/30	0	
	600 ^a	I.P.	30 minutes	12/14	86 (1 tox)	950
			1 hour	15/15	100	
			90 minutes	14/15	93	
			2 hours	15/15	100	
			3 hours	14/15	93	
			4 hours	6/15	40	
			5 hours	1/15	7	
			6 hours	2/15	13	
			—	0/40	0	
			700 ^a	Oral	1 hour	
	2 hours	6/10			60	
	3 hours	5/10			50	
	—	0/10			0	
950 rads (γ)	700 ^a	Oral	2 hours	13/15	87	1500
			3 hours	10/15	67	
			4 hours	2/15	13	
			5 hours	0/15	0	
			—	0/20	0	

^a pH 7.2 in distilled water.

TABLE 6
Radiation Protection in Large Animals. WR 2721:
 $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2\text{SPO}_3\text{H}_2$

Species	Radiation Dose	Drug Dose	30-Day Survival	
			Treated	Control
Dog	450 rads (x-ray)	200 mg/kg IV (30 minutes)	5/5 (1 tox)	0/5
Dog	650 rads (γ)	150 mg/kg IV (30 minutes)	8/16	0/6
Rhesus Monkey	1000 rads ^a (γ)	250 mg/kg IV (30 minutes)	6/6	0/2
Rhesus Monkey	1000 rads ^a (γ)	300 mg/kg Oral (30 minutes)	0/6	0/2

^a LD₅₀/30 days = 850 rads.

mice was evident to 3 hours. Orally, at a dose of 700 mg/kg, protection was also optimal at 1–2 hours and persisted to about 3 hours. Unfortunately, this oral protection has not been demonstrated in larger animals.

Both dogs and monkeys have been protected by WR 2721 administered I.V. Table 6 presents data demonstrating that protection.

During the program two different sources were employed for irradiating dogs and monkeys. The first was the van de Graaf accelerator at the NIH. In this facility, dose rates were approximately 100 rads of 2 meV x-rays per minute at a target to midline distance of 2 m. In this configuration the LD₅₀/30 days in dogs was 350 rads, and at 450 rads (the dose used for drug testing) mortality was about 95% among control dogs over several years of experiments.

The other facility used for irradiating large animals later in the program was the Triga Mark IV reactor. In this facility the geometry was arranged to deliver fission spectrum γ -radiation at a dose rate of approximately 100 rads per minute midline tissue dose, with neutrons excluded so that the neutron contribution to the total dose was less than 2%. In all cases animals receiving the protective chemicals were irradiated side-by-side with vehicle controls. As in other experiments that are described, the total dose delivered was intended to be just sufficient to produce 100% mortality in controls. In dogs, this dose was 650 rads, and in practice control mortality was 97% over several years. In monkeys the 95% lethal dose was 850 rads. Protection of dogs with WR 2721 was demonstrated in two experiments. At 200 mg/kg, a dose producing one toxic death, five of five dogs surviving the toxic effects of the drug, survived irradiation. At 150 mg/kg, a better tolerated dose, 8 of 16 dogs survived. Rhesus monkeys were protected by 250 mg/kg administered intravenously 30 minutes before irradiation, while orally, the compound did not protect at 300 mg/kg.

Radioprotective activity of WR 2529

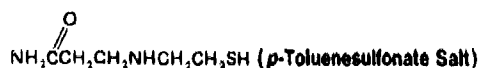
The first of the five compounds recommended for further study is WR 2529. This compound has a free sulfhydryl group, and the substituent on the nitrogen function is an amide. In all cases the compound was in the form of a *p*-toluenesulfonate salt.

TABLE 7
Radioprotective Activity of WR 2529 in ICR Mice

Radiation Dose	Drug Dose (mg/kg)	Route	Time (minutes)	Survivors	Percent Survival	Toxic LD ₅₀ (mg/kg)
1000 rads (γ)	900 ^a	I.P.	15	10/10	100	1100
	450			9/10	90	
	225			7/10	70	
	0			0/10	0	
	175 ^a			0/15	0	
	87.5	I.P.	15	0/15	0	
	0			0/10	0	
	2500 ^a		15	3/15	20	
	2500		30	5/15	33	
	0			0/10	0	

^a pH adjusted to 5.5 in distilled water.

TABLE 8
Radiation Protection in Large Animals. WR 2529:



Species	Radiation Dose	Drug Dose	30-Day Survival	
			Treated	Control
Rhesus	1200 rads ^a	400 mg/kg I.V.	2/6	0/6
Monkey	(γ)	(15 minutes)		
Miniature Swine	600 rads	300 mg/kg I.V.	8/11	0/12
	(γ)	(30 minutes)	(1 tox)	
Dog	Maximum tolerated dose 150 mg/kg. Protection study not attempted.			

^a Approximate LD_{50/30 days} = 850 rads.

WR 2529 (Table 7) was very well tolerated by mice. The I.P. LD₅₀ was 1100 mg/kg; and orally, the LD₅₀ was above 2500 mg/kg. Apparently the compound is not well absorbed, and oral protective activity was quite minimal.

Intraperitoneally, WR 2529, at a maximum tolerated dose (900 mg/kg), protected 100% of mice when administered 15 minutes before irradiation. At half the maximum tolerated dose (450 mg/kg) survival was 90%, and at one-quarter MTD, there was 70% survival. Little or no survival was observed at lower drug doses. Yuhas et al.¹² have reported a DRF of 2.6 for this compound in mice, and in terms

of this indicator of protective activity WR 2529 is second only to WR 2721 of the compounds developed by the Army program.

Both monkeys and miniature swine have been protected with WR 2529 (Table 8). Dogs exhibited an unusual sensitivity to this compound, tolerating only approximately 150 mg/kg I.V. Protection has not been attempted in the dog. In rhesus monkeys, two of six were protected against a 1200-rad dose. This is well above a just-lethal dose of radiation. In miniature swine, 8 of 11 animals given 300 mg/kg I.V. survived a just-lethal dose of radiation with one death due to drug toxicity.

TABLE 9
Radioprotective Activity WR 3689 in ICR Mice

Radiation Dose	Drug Dose (mg/kg)	Route	Time (minutes)	Survivors	Percent Survival	Toxic LD ₅₀ (mg/kg)
1000 rads (γ)	400 ^a	I.P.	15	15/15	100	1300
	200			13/15	87	
	100			7/15	47	
	50			2/15	13	
	0			0/20	0	
	1000 ^b	Oral	15	0/15	0	1700
			30	1/14	7	
			60	9/15	60	
			—	0/20	0	
	0		—	0/20	0	
975 rads (γ)	1000 ^b	Oral	60	10/15	67 (5 tox)	1700
	500			13/15	87	
	250			14/15	93	
	125			11/15	73	
	0			0/20	0	

^a pH 7.4 in phosphate buffer.

^b pH 6.5 in distilled water.

TABLE 10
Radiation Protection in Large Animals. WR 3689: $\text{CH}_3\text{NHCH}_2\text{CH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2\text{SPO}_3\text{H}_2$

Species	Radiation Dose	Drug Dose	30-Day Survival	
			Treated	Control
Dog	650 rads (γ)	400 mg/kg I.V. (30 minutes)	4/5 (4 tox)	0/3
Dog	650 rads (γ)	300 mg/kg I.V. (30 minutes)	4/8	0/3

Radioprotective activity of WR 3689

WR 3689 is a phosphorothioate differing from WR 2721 only by the addition of a terminal methyl group.

In mice (Table 9) WR 3689 was better tolerated than WR 2721 both I.P. and orally. The I.P. LD_{50} was 1300 mg/kg and the oral LD_{50} , 1700 mg/kg. The similarity between I.P. and oral LD_{50} 's suggests good oral absorption. The maximum tolerated dose

was 800 mg/kg, and at half that dose (400 mg/kg) survival was 100%. At the quarter-dose and eighth dose survival was 87% and 47%, respectively. It would appear that I.P. protection is not as good as with WR 2721 although 15 minutes may not be the optimal time of administration. The DRF has not been determined for this compound as yet. Orally WR 3689 showed definite superiority over WR 2721. Optimal oral protective activity did not develop until at least 60 minutes at a maximum

TABLE 11
Radioprotective Activity of WR 44923 in ICR Mice

Radiation Dose	Drug Dose (mg/kg)	Route	Time	Survivors	Percent Survival	Toxic LD_{50} (mg/kg)
950 rads (γ)	300 ^a	I.P.	15 minutes	30/30	100	550
	150			30/30	100	
	75			12/15	80	
	37.5			6/15	40	
	18.7			0/10	0	
	0			0/30	0	
	300 ^a	I.P.	2 hours	13/15	87	550
			3 hours	6/15	40	
			4 hours	1/15	7	
			5 hours	2/15	13	
			6 hours	0/15	0	
	0		—	0/30	0	
	700 ^a	Oral	1 hour	14/14	100	>1200
			2 hours	15/15	100	
			3 hours	12/15	80	
			4 hours	9/15	60 (3 tox)	
			5 hours	3/15	20	
			6 hours	2/15	13	
			7 hours	4/15	27	
			8 hours	2/15	13	
	0		—	0/40	0	
	350 ^a	Oral	30 minutes	8/15	53	>1200
	350		60 minutes	7/15	47	
	0		—	0/10	0	

^a pH 7.0 in methylcellulose/Tween-80 suspension.

tolerated dose of 1000 mg/kg. It was also noted that at a dose of only one-fourth the MTD (250 mg/kg) protection was still 93%; at one-eighth, 73% survival was observed.

Protection has also been demonstrated in the dog (Table 10). At a just-supralethal dose of γ -radiation (650 rads), a toxic dose of WR 3689 (400 mg/kg) produced four of five survivors although four dogs died of drug toxicity at this dose. At a lower dose (300 mg/kg) there was no toxic mortality, and four of eight treated dogs survived. No attempt has been made to protect dogs or monkeys by oral administration of WR 3689 as yet.

Radioprotective activity of WR 44923

WR 44923 is also a phosphorothioate, differing from WR 2721 in that there are three rather than two carbons between the nitrogen and sulfur.

In mice (Table 11), WR 44923 was not as well tolerated as WR 2721 by intraperitoneal administration; the I.P. LD₅₀ was only 550 mg/kg. At 15 minutes, there was 100% survival at the MTD and at half the MTD. At one-fourth the MTD (75 mg/kg) survival was 80%. Thus WR 44923 protected as well as WR 2721 at a dose of 75 mg/kg, but the therapeutic index was less at this level. The duration of protection after I.P. administration (2-3 hours) was similar to that observed with WR 2721. Orally, WR 44923 at the maximum tolerated dose (700 mg/kg) provided 100% survival for up to 2 hours and greater than 60% survival to 4 hours. Thus WR 44923 appears to perhaps have a slightly

TABLE 12

Radiation Protection in Large Animals. WR 44923:
NH₂CH₂CH₂CH₂NHCH₂CH₂CH₂SPO₃H₂

Species	Radiation Dose	Drug Dose	30-Day Survival	
			Treated	Control
Dog	650 rads (γ)	200 mg/kg I.V. (30 minutes)	7/8	0/3

longer duration of action than WR 2721. Protection at 350 mg/kg orally was only modest.

WR 44923 also protected dogs against just-supralethal irradiation (Table 12). At a dose of 200 mg/kg administered intravenously 30 minutes before exposure, seven of eight dogs survived.

Radioprotective activity of WR 77913

WR 77913 is a phosphorothioate of mercaptopypylamine. This is an unusual compound in that there is a hydroxyl group substituted on the middle carbon.

WR 77913 (Table 13) was exceptionally well tolerated in mice. The LD₅₀ I.P. was 1650 mg/kg. Orally the LD₅₀ was 4200 mg/kg, possibly suggesting poor absorption by this route.

Intraperitoneally, a dose equivalent to one-quarter MTD (200 mg/kg) protected 97% of the mice. The compound exhibited some protective activity orally, but even at high doses (1500 mg/kg) survival was no better than 67%. We have no in-

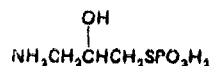
TABLE 13

Radioprotective Activity of WR 77913 in ICR Mice

Radiation Dose	Drug Dose (mg/kg)	Route	Time (minutes)	Survivors	Percent Survival	Toxic LD ₅₀ (mg/kg)
975 rads (γ)	800 ^a	I.P.	15	15/15	100	1650
	400			30/30	100	
	200			29/30	97	
	100			4/15	27	
	0			0/20	0	
	1500 ^a	Oral	30	10/15	67	4200
	750			3/15	20	
	0			0/10	0	
	1500 ^a	Oral	60	9/15	60	
	1500		90	10/15	67	
	0		—	0/10	0	

^a pH 7.8 in distilled water.

TABLE 14
Radiation Protection in Large Animals. WR 77913:



Species	Radiation Dose	Drug Dose	30-Day Survival	
			Treat ^a	Control
Dog	650 rads (γ)	680-720 mg/kg I.V. (30 minutes)	14/16	0/6

formation on the duration of action of this compound.

Excellent protective activity has been demonstrated in dogs with WR 77913 (Table 14). Dogs tolerated WR 77913 exceptionally well, and at a dose of 700 mg/kg 14 of 16 dogs survived just-supralethal irradiation.

Radioprotective activity of WR 168643

WR 168643 is a structurally unusual protective compound in that it has no nitrogen function. There are no data on the metabolism of this unusual

compound, and thus it is not known whether a free sulfhydryl group is released *in vivo* or not.

Data demonstrating protection of mice are presented in Table 15. Toxicity studies in mice with this compound have not been completed, and we know only that the LD₅₀ is above 950 mg/kg either I.P. or orally.

After administration of 300 mg/kg 15 minutes prior to irradiation 100% survival was observed. The 300 mg/kg dose was well tolerated, and this dose is apparently well below the maximum tolerated dose. A high percent survival was observed down to doses of 37.5 mg/kg, and even at 18.75 mg/kg, survival was 57%. Although we cannot quantitate the therapeutic index, it is clearly exceptionally high. The duration of protection is, however, very brief; and at 60 minutes, little or no protection was observed either I.P. or orally.

Orally, good protection was also obtained when WR 168643 was administered 15 minutes before irradiation. There was 100% survival after doses of 600 and 300 mg/kg, and 73% survival after 150 mg/kg.

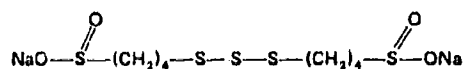
WR 168643 protected four of nine dogs against

TABLE 15
Radioprotective Activity of WR 168643 in ICR Mice

Radiation Dose	Drug Dose (mg/kg)	Route	Time (minutes)	Survivors	Percent Survival	Toxic LD ₅₀ (mg/kg)
975 rads (γ)	300 ^a	I.P.	15	15/15	100	>950
	150			14/15	93	
	75			13/15	87	
	37.5			32/40	80	
	18.75			20/35	57	
	9.38			0/10	0	
	0			0/30	0	
850 rads (x-ray)	300 ^a	I.P.	60	0/10	0	
	150			1/10	10	
	0			0/10	0	
975 rads (γ)	600 ^a	Oral	15	15/15	100	>950
	300			15/15	100	
	150			11/15	73	
	75			6/30	20	
	37.5			2/10	13	
	0			0/30	0	
850 rads (x-ray)	300 ^a	Oral	60	1/10	10	
	150			0/10	0	
	0			0/10	0	

^a pH 6.8 in distilled water.

TABLE 16
Radiation Protection in Large Animals. WR 168643:



Species	Radiation Dose	Drug Dose	30-Day Survival	
			Treated	Control
Dog	650 rads (γ)	100 mg/kg I.V. (30 minutes)	4/9	1/3
Rhesus Monkey	850 rads (γ)	200 mg/kg I.V. (30 minutes)	0/6	0/2

650 rads of γ -radiation in an experiment in which one of three control dogs survived (Table 16). As described above, this dose of radiation was 97% lethal overall among control dogs.

There was no survival among six rhesus monkeys given 200 mg/kg I.V. 30 minutes before irradiation. We believe that both dogs and monkeys will tolerate doses of WR 168643 higher than those administered in these experiments.

Comparative radioprotective properties of the several radioprotectors

Table 17 summarizes the radiation protection obtained in mice after intraperitoneal administration of the compounds discussed.

The lowest dose of each drug required to produce at least 50% survival (A) is listed, and the toxic dose (B) is expressed as an LD₅₀. The therapeutic index presented is defined as the ratio of the toxic LD₅₀ dose (B) divided by the minimum 50% protective dose (A). The duration of protection is de-

fined as the longest time at which greater than 50% survival was obtained. Also indicated is the DRF which Yuhas and Storer (private communication) obtained in C₅₇B1/6J mice against 30-day mortality.

The older radioprotectors, MEA and AET, were more toxic than the newer radioprotectors and had low therapeutic indices (only 1.3 and 2.2, respectively). The duration of protection was rather brief, and the DRF's were only 1.6. The highest DRF (2.7) has been obtained with WR 2721. This compound also had a therapeutic index of 12 and a 3-hour duration of protection.

WR 2529, the amide, which has no sulfhydryl covering function, had a DRF of 2.6—second only to WR 2721 in this respect. The protective dose of WR 2529 (225 mg/kg) was higher than that of WR 2721 (75 mg/kg), and although WR 2529 was slightly less toxic, its therapeutic index (4.9) was smaller. WR 2529 protected only for a relatively brief period (15–30 minutes).

The other three phosphorothioates in this group of compounds—WR 3689, WR 44923, and WR 77913—had good protective activity by intraperitoneal administration, although none of these three compounds had as good a therapeutic index as WR 2721. WR 44923 had a DRF of 1.8; the DRF's of the other two compounds are not known. WR 44923 protected at 75 mg/kg as did WR 2721, and it protected for 3 hours, as did WR 2721. WR 168643, the compound with no nitrogen function, protected more than 50% of mice at a dose of only 18.75 mg/kg, and had a therapeutic index above 50. The duration of protection provided by WR 168643 was brief. Its DRF is unknown.

TABLE 17
Summary of Mouse Protection (I.P. Administration)

Compound	(A) mg/kg Dose to Protect >50%	(B) Toxic LD ₅₀ (mg/kg)	B ÷ A Therapeutic Index	Duration of Protection	DRF ^a
MEA	150	200	1.3	15 minutes	1.6
AET	112.5	250	2.2	60 minutes	1.6
WR 2721	75	900	12.0	3 hours	2.7
WR 2529	225	1100	4.9	15–30 minutes	2.6
WR 3689	200	1300	6.5	Unknown	Unknown
WR 44923	75	550	7.3	3 hours	1.8
WR 77913	200	1650	8.2	Unknown	Unknown
WR 168643	18.75	>950	>50	15 minutes	Unknown

^a Storer and Yuhas in C57B/6J mice.

TABLE 18
Summary of Mouse Protection (Oral Administration)

Compound	(A) mg/kg Dose to Protect >50%	(B) Toxic LD ₅₀	B ÷ A Therapeutic Index	Duration of Protection
MEA	No protection	700	—	—
AET	250	600	2.4	30-60 minutes
WR 2721	700	1500	2.1	3 hours
WR 2529	Weak protection	>2500	—	—
WR 3689	125	1700	13.6	Unknown (>60 minutes)
WR 44923	300	>1200	>4	4 hours
WR 77913	1500	4200	2.8	Unknown (>90 minutes)
WR 168643	150	>950	>6.3	15 minutes

Table 18 summarizes the oral protective activity of these compounds in the mouse. MEA was not protective orally in the mouse, and although AET protected orally, its therapeutic index was only 2.4, and the duration of protection was only 30-60 minutes.

WR 2721 also protected orally, and although the therapeutic index was low (2.1) the duration of protection extended to 3 hours.

The thiol, WR 2529, had only weak protective activity orally, probably because of poor oral absorption. The phosphorothioate, WR 77913, protected orally, but only at high doses and its therapeutic index was low.

The other two phosphorothioates—WR 3689 and WR 44923—appear to have oral protective activity superior to WR 2721. WR 3689 protected orally at a dose of only 125 mg/kg, and had a therapeutic index of 13.6. WR 44923 protected orally at a dose of 300 mg/kg, and its therapeutic index was greater than 4. The duration of oral protection afforded by WR 44923 was 4 hours, slightly longer than WR 2721.

WR 168643, the non-nitrogen compound, protected orally for only a relatively brief period. The minimum protective dose was 150 mg/kg, and its therapeutic index was greater than 6.3.

Table 19 summarizes the protective activity of these compounds in larger animals.

Neither MEA nor AET protected dogs in our laboratory. We have not tested MEA or AET in monkeys in our laboratory, but others have reported no protection in dogs with AET,³ and protection in monkeys with AET only at doses which are associated with severe toxicity.⁵

WR 2721 and all of the five recommended

compounds have all been demonstrated to protect at least one species of large animals by intravenous administration.

WR 2529 was too toxic in dog tolerance studies to suggest that the dog could be protected, and such a study has not been attempted. WR 2529, however, has protected both rhesus monkeys and miniature swine.

The three phosphorothioates—WR 3689, WR 44923, and WR 77913—all protected dogs by intravenous administration, and no monkey studies have been conducted. WR 2721 induced vomiting in the dog at oral doses below those that we believe would be required to protect, and thus oral protection has been attempted only in the rhesus monkey. In one experiment, monkeys were not protected orally. Oral protection has not been attempted in dogs or monkeys with either WR 3689 or WR 44923, the two compounds with superior oral protective activity in mice; but these studies will be undertaken in the near future.

TABLE 19
Radioprotective Activity in Large Animals
(Intravenously)

Compound	Dog	Rhesus Monkey
MEA	No	No
AET	No	Yes (with toxicity)
WR 2721	Yes	Yes (not orally)
WR 2529	Too toxic	Yes (also protects swine)
WR 3689	Yes	
WR 44923	Yes	
WR 77913	Yes	
WR 168643	Yes	No (at less than MTD)

WR 168643 protected dogs at a well-tolerated dose, but monkeys were not protected in a single experiment. Further experiments will be conducted to determine whether a higher I.V. dose or an oral dose would confer protection.

Summary and conclusions

In conclusion, we recommend that further studies be pursued with five radioprotective compounds developed by the Army program. One of these, (WR 2529) has a DRF of 2.6, which approaches that of WR 2721.

The phosphorothioate WR 77913 is exceptionally well tolerated in mice and dogs, and particularly in dogs it appears to confer excellent protection of modest duration after parenteral administration.

Two other phosphorothioates (WR 3689 and WR 44923) appear to have protective activity superior to WR 2721 after oral administration to mice.

WR 168643 is a novel sulfur compound having no nitrogen function. It has excellent protective activity at low doses in the mouse, and it is well tolerated by mice, dogs, and monkeys. Its protective activity appears to be of brief duration. Protection has been observed in dogs after intravenous administration.

The objective of the Army program was and is whole body protection by oral administration. For the most part, the radioprotective activity of the recommended compounds has been assessed only against 30-day mortality, and against radiation damage to hematopoietic tissues. Whether these newer compounds will protect normal tissues better than solid tumors is not known, and the extent of protection that can be expected in a variety of radiosensitive normal tissues has yet to be studied. We recommend that these studies be done in laboratory animals to determine whether any of these compounds might have clinical potential in radiotherapy.

References

1. Alexander, P., Bacq, Z. M., Cousens, S. F., Fox, M., Herve, A., and Lazar, J.: Mode of action of some sub-

stances which protect against the lethal effect of x-rays. *Radiat Res* 2: 392-415, 1955.

2. Bacq, Z. M., and Alexander, P.: *Fundamentals of Radiobiology* (2nd ed.). Pergamon Press, New York, 1961.
3. Benson, R. E., Michaelson, S. M., Downs, W. L., Maynard, E. A., Scott, J. K., Hodge, H. C., and Howland, J. W.: Toxicological and radioprotection studies on S,β-aminoethylisothiuronium bromide (AET). *Radiat Res* 15: 561-572, 1961.
4. Carroll, F. I., Dickson, H. M., and Wall, M. E.: Organic sulfur compounds. III. Synthesis of 2-(substituted alkylamino)ethanethiols. *J Organ Chem* 30: 33-38, 1965.
5. Crouch, B. G., and Overman, R. R.: Chemical protection against x-irradiation death in primates; a preliminary report. *Science* 125: 1092, 1957.
6. Doherty, D. G., Burnett, W. T., and Shapira, R.: Chemical protection against ionizing radiation. II. Mercaptoalkylamines and related compounds with protective activity. *Radiat Res* 7: 13-21, 1957.
7. Eldjarn, L., and Pihl, A.: On the mode of action of x-ray protective agents. I. The fixation *in vivo* of cystamine and cysteamine to proteins. *J Biol Chem* 224: 341-352, 1956.
8. Hollaender, A.: *Radiation Protection and Recovery*. Pergamon Press, New York, 1960.
9. Piper, J. R., Stringfellow, C. R., Elliot, R. D., and Johnson, T. P.: S-2-(ω-aminoalkylamino)ethyl dihydrogen phosphorothioates and related compounds as potential antiradiation agents. *J Med Chem* 12: 236-243, 1969.
10. Piper, J. R., Stringfellow, C. R., and Johnson, T. P.: Terminally substituted S-2-(aminoalkylamino)ethyl dihydrogen phosphorothioates and related compounds as potential antiradiation agents. *J Med Chem* 12: 244-253, 1969.
11. Srivastava, P. K., and Field, L.: Organic disulfides and related substances. 38. Some disulfide and trisulfide sulfinate salts as antiradiation drugs. *J Med Chem* 18: 798-802, 1975.
12. Yuhas, J. M.: Biological factors affecting the radioprotective efficiency of S-2-(3-aminopropylamino)-ethyl phosphorothioic acid (WR 2721). LD₅₀₍₃₀₎ doses. *Radiat Res* 44: 621-628, 1970.
13. Yuhas, J. M.: Improvement of lung tumor radiotherapy through differential chemoprotection of normal and malignant tissues. *J Natl Cancer Inst* 48: 1255-1257, 1972.
14. Yuhas, J. M.: Radiotherapy of experimental lung tumors in the presence and absence of a radioprotective drug, S-2-(3-aminopropylamino)ethyl phosphorothioic acid (WR 2721). *J Natl Cancer Inst* 50: 69-78, 1973.

